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Ruminal Protein Degradation Characteristics of Cell Mass from Lysine Production

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ABSTRACT : Chemical analysis and *in vitro* studies were conducted to investigate the nutritive value for ruminants of cell mass from lysine production (CMLP) which is a by-product of the lysine manufacturing process. Proximate analysis, protein fractionation, and *in vitro* protein degradation using protease from *Streptomyces griseus* and strained ruminal fluid were carried out to estimate ruminal protein degradability of CMLP with two reference feedstuffs-soybean meal (SBM) and fish meal (FM). Amino acid composition and pepsin-HCl degradability were also determined to evaluate postruminal availability. CMLP contained 67.8% crude protein with a major portion being soluble form (45.4% CP) which was composed of mainly ammonium nitrogen (81.8% soluble CP). The amount of nucleic acids was low (1.15% DM). The total amount of amino acids contained in CMLP was 40.60% DM, which was lower than SBM (47.69% DM) or FM (54.08% DM). CMLP was composed of mainly fraction A and fraction B2, while the protein fraction in SBM was mostly B2 and FM contained high proportions of B2 and B3 fractions. The proportion of B3 fraction, slowly degradable protein, in CP was the highest in fish meal (23.34%), followed by CMLP (7.68%) and SBM (1.46%). CMLP was degraded up to 51.40% at 18 h of incubation with *Streptomyces* protease, which was low compared to FM (55.23%) and SBM (83.01%). This may be due to the insoluble portion of CMLP protein being hardly degradable by the protease. The *in vitro* fermentation by strained ruminal fluid showed that the amount of soluble fraction was larger in CMLP (40.6%) than in SBM (17.8%). However, because the degradation rate constant of the potentially degradable fraction of CMLP (2.0%/h) was lower than that of SBM (5.8%/h), the effective ruminal protein degradability of CMLP (46.95%) was slightly lower than SBM (53.77%). Unavailable fraction in the rumen was higher in CMLP (34.0%) compared to SBM (8.8%). *In vitro* CP degradability of CMLP by pepsin was 80.37%, which was lower than SBM (94.42%) and FM (89.04%). The evaluation of protein degradability using different approaches indicated that soluble protein in CMLP may supply a large amount of ammonia in the rumen while insoluble protein can be by-passed from microbial attacks due to its low degradability. The results from this study suggest that CMLP can be used as a protein supplement to ruminants for supplying both non-protein nitrogen to rumen microbes and rumen undegradable protein to the host animal. (**Key Words** : Cell Mass from Lysine Production, Ruminal Protein Degradability, Protein Fraction)

INTRODUCTION

Feed cost accounts for 50 to 70% of animal production. In an economical perspective, thus, reducing the feed cost is the most critical for efficient animal production. Protein sources in animal feeds are one of the most expensive ingredients and the price of high quality protein feeds has been continuously increasing (Piao et al., 1998; Kondo et al., 2007). Given this situation, there have been many attempts in the cattle industry to find less expensive protein sources which can replace conventional and more expensive protein feeds such as soybean meal and fish meal. Some of these approaches have involved the recycling of industrial wastes to produce supplemental protein sources, such as pulp mill wastes (Kellems et al., 1981), brewers single cell protein (Johnson and Remillard, 1983), potato-corn biosolids single cell protein (Hsu et al., 1984), bacterial single cell protein (Sedgman et al., 1985a; Sedgman et al., 1985b), poultry by-product meal (Bohnert et al., 1999) and other types of by-products(Wanapat et al., 2006; Kumar et al., 2007). The recycling of industrial waste products into animal feed can also reduce environmental concerns.

During the manufacturing process of lysine, which is one of the most important synthetic amino acids supplemented to farm animal diets, several fermentation by-

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products can be produced. Condensed molasses solubles (CMS), for instance, has been evaluated in ruminant animals by many researchers (Hannon and Trenkle, 1990; Kim et al., 1997; Ha et al., 1998; Lee et al., 1998; Broderick et al., 2000; Liu and McMeniman, 2001). Besides CMS, bacterial cell mass is also produced during lysine fermentation. Cell mass from lysine production (CMLP), which is commercially available, is mainly composed of dried bacterial cell mass (Corynebacterium) and may be used as a protein source in animal diets. There was a study showing that CMLP can be an alternative high protein ingredient for broiler chicks in the starter period (Piao et al., 1998). For ruminants, however, no investigation of the nutritive value of CMLP has been reported. The protein digestive system in ruminants is quite different from non-ruminants because of active microbial fermentation in the rumen. In the metabolisable protein (MP) system of ruminants it is necessary to account for both rumen degradable and undegradable fractions to supply adequate amounts of protein to the animal. Rumen degraded protein is taken up by the rumen bacteria and converted into microbial protein while the undegraded fraction can be digested and absorbed at the lower digestive tract and be an amino acid source to the animal. A preliminary study showed that this new feed contained more than 60% crude protein and a large amount of both rumen degradable and undegradable proteins. The readily degradable nitrogen was mainly ammonium salt added during the process with the undegradable part being bacterial true protein. Thus, CMLP may supply both non-protein-nitrogen (NPN) to the bacteria and rumen undegradable protein (RUP) to the animal.

The aim of this study was to investigate the potential value of cell mass from lysine production as a protein source for ruminants using various chemical and biological methods.

MATERIALS AND METHODS

Chemical analysis, protein fractionation, enzymatic degradation, and *in vitro* ruminal fermentation using strained ruminal fluid were used to investigate the value of CMLP as a protein source. CMLP was kindly provided by BASF, Korea and soybean meal (SBM) and fish meal (FM) were purchased from a local feed company. All feed samples were ground through a 1mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) before use in various chemical analysis and *in vitro* tests.

Chemical analysis

Nutrient composition : All chemical analyses for nutrients of CMLP were performed with two reference feeds; SBM and FM. Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash were analyzed according to AOAC (1984) and NDF, ADF and lignin were determined using the method of Van Soest et al. (1991).

Protein fractionations : Test feedstuffs were analyzed for non-protein-nitrogen, buffer soluble nitrogen, neutral-detergent insoluble nitrogen and acid-detergent insoluble nitrogen, and then they were fractionated into 5 fractions as described by Licitra et al. (1996). Ammonium nitrogen (Chaney and Marbach, 1962) and nucleic acid nitrogen (Zinn and Owens, 1986) were measured. The amino acid composition of each feedstuff was also determined with an Amino Acid Analyzer (L-8500A, Hitachi, Japan). Proteins in the CMLP, SBM, and FM were partitioned into five fractions as proposed by Sniffen et al. (1992). Fraction A is non-protein-nitrogen and is rapidly converted into ammonia in the rumen. Fraction B is classified as available true protein and subdivided into three fractions, B1, B2, and B3, based on their rate of degradation in the rumen. B1 fraction is buffer-soluble but TCA-precipitable and is rapidly degraded in the rumen. B2 is soluble in neutral detergent but insoluble in borate-phosphate buffer fraction and is ranked intermediate in the rate of degradation among the B fractions. B3 fraction is soluble in acid detergent but insoluble in neutral detergent and is slowly degraded in the rumen because of its association with the cell wall. C fraction is insoluble in acid detergent and theoretically unavailable to the animal.

Protein degradability by microbial protease

The enzymatic estimation of protein degradability with protease from *Streptomyces griseus* was performed as suggested by Licitra et al. (1998, 1999); however, periodical incubation instead of a single time-point technique was used. Each feedstuff (0.5 g) was incubated in borate-phosphate buffer (pH 6.7-6.8; $NaH_2PO_4 \cdot H_2O$, 12.20 g/L; $Na_2B_4O_7 \cdot 10H_2O$, 8.91 g) with protease from *Streptomyces griseus*, type XIV (4.6 units/mg protein, Sigma Chemical Co). The protease solution was prepared to contain 0.33 units of enzyme/ml in borate phosphate buffer; therefore, the final concentration of protease in the buffer solution was approximately 0.66 units/ml. The volume of enzyme solution to be added was calculated as described by Licitra et al. (1998). The incubation was continued for 6, 12, 18 and 24 h at 39°C. After incubation, the solution was immediately filtered on Whatman #541 paper. The residue together with filter paper was transferred into a Kjeltec flask, dried in a 70°C oven overnight, and the nitrogen content was measured by a Kjeltec Auto 1035/1038 system (Tecator, Sweden).

Protein degradability by strained rumen fluid

In vitro crude protein degradability of CMLP and SBM was determined using strained ruminal fluid (SRF) as

inoculum. Urea was also incubated as a blank. A basal buffer solution which is known as artificial medium was used for maintaining adequate pH during the incubation. The ratio of ruminal fluid to buffer solution was the same as suggested by Menke and Steingass (1988), except that 0.2 g of glucose and cellobiose were added to 100 ml medium to supply sufficient carbohydrate source. The rumen fluid inoculum was obtained from a Holstein steer weighing about 600 kg in which a permanent ruminal fistula was fitted. The steer received a mixture of 50% timothy hay and 50% concentrate (16% CP) twice daily to meet maintenance requirements. The rumen fluid was collected in the morning before feeding, filtered through two layers of cheese cloth, and maintained without either oxygen or temperature shock. The samples containing the same amount of CP as 0.3 g of SBM (CMLP, 0.201 g; urea, 0.042 g) were weighed into 125 ml serum bottles. With a strictly anaerobic technique, 5 ml of rumen fluid and 30 ml of *in vitro* medium were added into the bottles which were sealed with butyl rubber stoppers and aluminum caps. The incubations were conducted in triplicate for 2, 4, 8, 12, 24, 48 and 72 h in a 39°C shaking incubator (60 rpm). Total gas produced during incubation was vented through a modified Bunsen gas release valve described by Tilley and Terry (1963), to prevent accumulation of gas and pressure.

At the end of each incubation time, the pH was measured. Tubes were then centrifuged immediately at 4°C for 15 min. at 14,000 g. The supernatant was transferred into a 1.5 ml microcentrifuge tube and stored at -20°C until ammonia nitrogen analysis as described by Chaney and Marbach (1962). The pellet and the remaining supernatant were filtered through Whatman #541 filter paper and residual nitrogen in the pellet material was measured by a Kjeltec Auto 1035/1038 system (Tecator, Sweden).

The disappearance of CP at each incubation time was determined by subtracting the residual CP from the original CP and was corrected for urea to minimize underestimation of protein degradation due to bacterial contamination. Data were then fitted to the equation of Ørskov and McDonald (1979) :

$$P = a+b(1-e^{-ct})$$

Where, P (% CP) is CP disappearance at time t (h), a (% CP) is soluble CP fraction, b (% CP) is insoluble but potentially degradable CP fraction, and c (h^{-1}) is a fractional rate of digestion.

Parameters were estimated by least-square methods using a nonlinear regression procedure of the SAS Institute (2002). *In vitro* effective CP degradability (ECPD) of feed samples was also calculated using the equation of Ørskov and McDonald (1979) :

$$ECPD = a+b\ (c/(c+k))$$

Where, ECPD is effective crude protein degradability, a, b and c are the same as above, and k is a fractional rate of passage out of the rumen, which was assumed as 0.06 h^{-1}.

Pepsin-HCl digestibility

Protein digestibility, using the pepsin-HCl procedure described by AOAC (1984), was also determined with some modification as proposed by England et al. (1997) to predict post-ruminal protein digestibility as follows. A total of 0.5 g of fat-extracted feed by a Soxtec system HT6 (Tecator, Sweden) was weighed into a 250 ml Erlenmeyer flask and 150 ml of pepsin-HCl (0.2% (w/v); activity, 1:10,000, prewarmed at 45°C) was added to the flask, which was capped with a marble and incubated with shaking for 16 h in a 45°C water bath. After incubation, the solution was filtered on a Whatman #541 paper. The residue and paper was transferred into a Kjeltec flask after drying in a 70°C

Table 1. Chemical composition and protein fractionations of tested feedstuffs

Composition	SBM	FM	CMLP
Dry matter (% DM)	89.18±0.96	92.33±3.16	89.39±1.63
Organic matter (% DM)	92.73±0.21	77.39±0.94	94.84±0.34
Crude fiber (% DM)	4.80±0.55	0.95±0.07	0.15±0.02
Ether extract (% DM)	2.02±0.10	7.55±0.96	5.81±0.39
Crude ash (% DM)	7.27±0.21	22.61±0.94	5.16±0.34
Crude protein (% DM)	48.62±0.62	58.41±1.11	67.68±1.01
NPN (% CP)[1]	8.75±1.01	22.26±1.20	39.88±0.49
TP (% CP)	91.25±1.01	77.74±1.20	60.12±0.49
SOLP (% CP)	15.75±1.63	22.28±0.15	45.38±1.15
NDIP (% CP)	2.57±0.37	29.39±5.73	10.98±1.77
ADIP (% CP)	1.11±0.61	6.06±1.18	3.30±0.53
NuA-N (% CP)	1.85±0.13	6.79±0.03	2.29±0.42
NH_3-N (% CP)	0.06±0.05	5.57±0.30	37.14±1.45

[1] NPN = Non protein nitrogen; TP = True protein; SOLP = Soluble protein; NDIP = Neutral detergent insoluble protein. ADIP = Acid detergent insoluble protein; NuA-N = Nucleic acid nitrogen; NH_3-N = Ammonium nitrogen.

Table 2. Amino acid composition of tested feedstuffs

	SBM		FM		CMLP
	% DM	% CMLP	% DM	% CMLP	% DM
Cys	0.805	365.3	0.540	245.2	0.220
Met	0.601	107.0	1.255	223.5	0.562
Asp	5.537	114.9	5.431	112.7	4.819
Thr	2.005	85.4	2.611	111.3	2.347
Ser	2.642	148.5	2.730	153.5	1.779
Glu	9.431	139.1	8.291	122.3	6.780
Gly	2.043	101.5	4.682	232.5	2.014
Ala	2.080	45.3	3.997	86.9	4.597
Val	1.969	90.9	2.362	109.1	2.166
Ile	1.904	105.6	1.991	110.4	1.803
Leu	3.793	106.1	4.261	119.1	3.576
Tyr	1.754	133.2	1.805	137.1	1.317
Phe	2.608	139.4	2.499	133.5	1.872
Lys	3.188	156.8	3.876	190.7	2.033
His	1.443	141.1	1.443	141.1	1.022
Arg	3.346	162.7	3.298	160.4	2.056
Pro	2.538	155.3	3.003	183.8	1.634
Total	47.688	117.5	54.076	133.2	40.597

Table 3. Protein fractions of tested feedstuffs based on chemical analysis and Cornell Net Carbohydrate and Protein System (CNCPS) (%CP)

	SBM	FM	CMLP
Protein fraction A	8.75±1.01	22.26±1.20	39.88±0.49
Protein fraction B1	7.00±2.64	0.02±1.35	5.50±1.64
Protein fraction B2	81.68±2.00	48.32±5.88	43.64±2.92
Protein fraction B3	1.46±0.98	23.34±6.91	7.68±2.3
Protein fraction C	1.11±0.61	6.06±1.18	3.30±0.53

oven overnight. The nitrogen content was then measured by a Kjeltec Auto 1035/1038 system (Tecator, Sweden).

Statistical analysis

All data were subjected to analysis of variance using a GLM procedure (SAS Institute, 2002) and differences between means were tested by LSD. The degradation kinetics were fitted by a non-linear regression using the Marquardt method of the NLIN procedure (SAS Institute, 2002); the statistical significance of differences in the parameter estimates between the tested feedstuffs was analyzed using a simple one-tailed student t-test.

RESULTS

Chemical analysis

Chemical composition, nitrogen fraction, and amino acid contents of SBM, FM, CMLP are shown in Table 1 and 2. CMLP contained a relatively large amount of NPN (39.88% CP) and soluble protein fraction (45.38% CP) compared to soybean meal (8.75 and 15.75% CP) and fish meal (22.26 and 22.28% CP). The majority of soluble crude protein of CMLP was ammonia nitrogen (81.8% soluble CP). CMLP also contained a higher content of NDIP

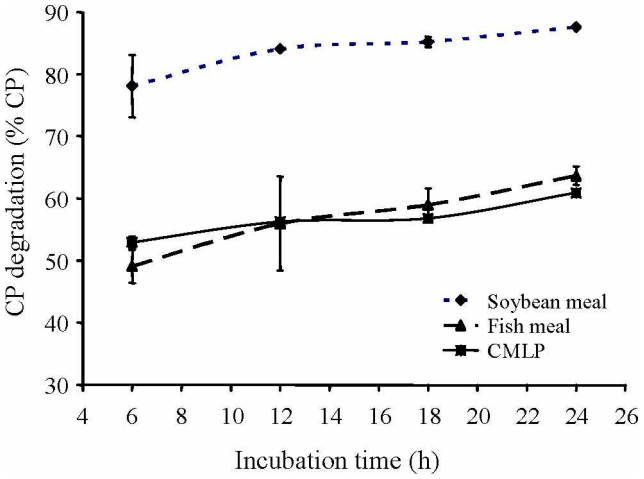

Figure 1. Crude protein degradation by *in vitro* incubation with protease from *S. griseus* (% CP).

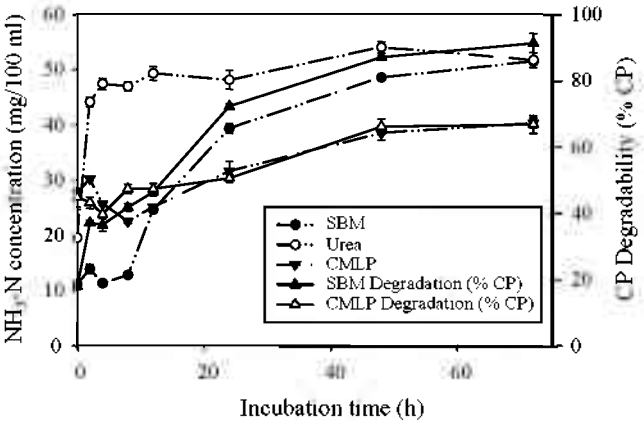

Figure 2. Changes in concentration of ammonium nitrogen and protein degradation during *in vitro* fermentation using strained rumen fluid.

(10.98% CP) than soybean meal (2.57% CP). The nucleic acid level of CMLP (1.55% DM) was higher than that of SBM but lower than that of FM (Table 1). Total amounts of amino acids in SBM, FM, and CMLP were 47.69, 54.08 and 40.60%, respectively, on a dry matter basis and 98.08, 92.58 and 60.60% on a crude protein basis. The content of individual amino acids of CMLP on a dry matter basis was relatively and numerically lower than that of soybean meal and fish meal except for alanine (Table 2).

The five protein fractions, which were determined according to CNCPS (Sniffen et al., 1992), of each feedstuff are shown in Table 3. CMLP was composed of mainly fraction A and fraction B2, while SBM protein was mostly B2 fraction. Fraction B3, which is a slowly degradable protein fraction, was the highest in fish meal (23.34% CP), followed by CMLP (7.68% CP) and SBM (1.46% CP).

Crude protein degradability

Degradation characteristics of SBM, FM, and CMLP by

Table 4. Kinetic parameters from *in vitro* ruminal protein degradation using strained rumen fluid

	SBM	CMLP	SEM[1]
Soluble protein** (% CP)	23.3	41.6	3.1
Potentially degradable protein (% CP)	72.7	46.9	28.3
Degradation rate constant* (L/h)	0.041	0.012	0.013
RMSE[2]	5.35	3.39	
R-squared[3]	0.97	0.92	
ECPD (% CP)[4]	52.8	49.4	

[1] SEM = Standard error of means. [2] RMSE=root mean square error. [3] R-squared = Coefficient of determination.
[4] ECPD = Effective CP degradability when passage rate was assumed to be 0.06 L/h. * $p<0.05$, ** $p<0.01$.

S. griseus protease are shown in Figure 1. CMLP showed similar crude protein degradability to FM, however, it was lower than SBM.

During *in vitro* incubation by strained rumen fluid, pH was not significantly different among the treatments (6.82±0.15). The concentration of ammonia nitrogen in the solution and CP degradability of tested feeds are shown in Figure 2. At time 0, when no digestion occurred, the concentration of ammonia in CMLP treatment was the highest because of its high content of ammonia nitrogen. However, subsequently the rate of ammonia production was slower than that of urea and SBM treatments. On the contrary, the ammonia concentration in the SBM treatment was low at the beginning but increased to the same level as the other treatments. In the urea treatment, all of the urea was converted to ammonia within 4 h.

CP degradability of tested feeds showed a correlation with the production of ammonia nitrogen. Protein degradation of CMLP was higher initially but lower than that of SBM during the latter part of incubation. Protein fractions, digestion rate constants, and effective CP degradabilities are presented in Table 4. The soluble fraction of CMLP in the *in vitro* study was similar to the value obtained by chemical fractionation (41.6% and 45.38%) and significantly higher than that of SBM ($p<0.01$). However, the value of unavailable fraction obtained from the *in vitro* incubation study (11.5% CP) was higher than that from chemical analysis (fraction C, 3.30% CP). This may indicate that some of the available CMLP protein which was determined chemically (fraction B) is resistant to degradation by microbial enzymes in the rumen. A similar tendency was observed in the degradation measured by a commercial protease as described above. Although CMLP contained a significantly larger amount of soluble protein fraction, no significant difference was observed in the effective CP degradability calculated from integration of a digestion rate with a passage rate between the two feedstuffs (Table 4). This could be explained by the numerically lower amount of insoluble but potentially degradable protein fraction of CMLP (46.9%) and the significantly slower digestion rate (0.012 h^{-1}) than those parameters for SBM (72.7% and 0.041 h^{-1}, respectively). *In vitro* CP degradability of CMLP by pepsin (data not shown) was 81.12±1.09 (% CP), which was lower than that of SBM (94.13±1.87) and FM (89.51±0.98) ($p<0.001$).

DISCUSSION

Chemical analysis

CMLP was composed of a large portion of protein fractions A and B1. This implies that CMLP contains a large amount of highly degradable CP in the rumen. Since bacteria that ferment structural carbohydrate (SC) use mainly ammonia as a N source (Russell et al., 1992), abundant ammonia nitrogen in CMLP could enhance SC bacterial growth when adequate carbohydrate is supplied. In addition, CMLP has more B3 fraction than SBM (Table 3). Fraction B3 is calculated by subtraction of ADIP from NDIP and is classified as a protein fraction which is insoluble but slowly degradable in the rumen (Sniffen et al., 1992), and thus a higher fraction B3 could reflect by-pass property of a feedstuff. Thus, CMLP seems to have a larger amount of RUP than SBM. Ruminal-escapable protein is important in dairy cattle nutrition because most common feedstuffs contain low digestible RUP (NRC, 2001). Reliance on feed proteins with high content of digestible RUP is greatest in high producing cows when most or all of the forage is provided by high quality grasses and legumes (NRC, 2001). In the case of CMLP, a high content of fraction B3 may be due to heat treatment during the production process. It is generally accepted that heat processing decreases rumen protein degradability by denaturation of proteins and by the formation of protein-carbohydrate (Maillard reactions) and protein-protein cross links. It is assumed that protein fraction B3 has an intestinal digestibility of 80% (NRC, 2001; Fox et al., 2004), but over-heating of feeds (i.e., heat-damaged protein) reduces the intestinal digestibility of RUP through the formation of indigestible Maillard products and protein complexes (Van Soest, 1994). Eventually, the higher the amount of fraction B3 in a feedstuff, the more proteins will flow into the intestine and be taken up and metabolized by the host animal. Our result of protein fractionation suggests that CMLP could act both as ruminal-degradable protein for microbial growth and as a RUP source for host ruminant animals.

The use of bacterial single cell protein (SCP) as a protein feed is sometimes limited because of its high content of nucleic acids. Nucleic acid content of bacterial SCP is usually in the range of 15-16% on a dry matter basis depending on the substrate (Anupama and Ravindra, 2000). Since the amount of nucleic acids in CMLP (Table 1) is relatively low, its content would not be a limitation for the use of CMLP as a protein source.

Crude protein degradability

Even though CMLP contained about 40% of protein that is soluble in the rumen, ECPD from *in vitro* fermentation using SRF was similar or less than SBM since CMLP had a large amount of undegradable protein and its degradation rate was low compared to SBM (Table 4). It was found that CMLP contained a higher content of undegradable protein in an *in vitro* rumen study (11.5%) and an enzymatic degradation study (39%), although the value assumed from chemical analysis (fraction C, 3.30%) was low. Furthermore, supposing that the undegradable fraction is residue after 72 h of incubation (Krishnamoorthy et al., 1983), it could be increased to 34.0% based on the *in vitro* trial (Figure 2). The inconsistency could be due to low digestibility of cell mass by microbial enzymes. This implies that chemical analysis using detergent solubility would not correctly estimate degradation or digestion which occurred in a biological process. Degradation not by chemical but by fungal protease would support this idea (Figure 1). CMLP contained a smaller amount of neutral-detergent insoluble nitrogen than FM (10.98% vs. 29.39% CP); however, CMLP was less degradable (60.91% vs. 63.67%) after 24 h of incubation.

Enzymatic procedures seemed to overestimate protein degradability compared to the *in vitro* technique using SRF at each time point (Figures 1 and 2), especially in early incubation. For example, protein degradability after 12 h of SBM was 46.3% using SRF and 81.7% using protease and that of CMLP was 47.2% and 50.8%, respectively, and so the difference was larger in SBM. This might be due to the existence of lag in an *in vitro* incubation. Even though there is also a lag phase in an enzymatic procedure (Licitra et al., 1999), live microbes may need more time to adjust to a new environment; therefore, some mathematical models describing *in vitro* gas production often contain a discrete lag in the equation (France et al., 1993; Schofield et al., 1994).

Figure 2 shows that CP degradability and ammonia N concentration follow a similar tendency, which may be indicative of the possibility of using ammonium concentration for CP degradation estimation. Using an electrode could have an advantage since this system can be easily automated. Further studies are needed.

To estimate ECPD we assumed a passage rate as 0.06 h^{-1} for SBM and CMLP throughout the studies. However, each feed may have its own passage rate according to a physical property, for example particle size and weight, or other factors including level and frequency of feeding may be involved (Sniffen et al., 1992; NRC, 2001). The passage rate constant of CMLP could be higher because of its small particle size. In a mathematical integration of digestion and passage rate constants, a higher passage rate constant results in a lower digestibility. Therefore, there is a possibility that CMLP under *in vivo* condition could have an even lower CP degradability than the values we obtained in the present experiment. Results from a pepsin degradability procedure implied that CMLP could be fairly well digested in the lower gut, though the amount may be less than SBM and FM.

The results from this study suggest a possible the use of CMLP to supply both NPN to rumen microbes and RUP to ruminant animals due to its unique chemical and physical properties. The values obtained in this study can be used to formulate an animal diet using CMLP which has economic benefit. Moreover, the techniques applied in the experiment, chemical analysis, enzymatic technique and *in vitro* degradation using SRF, would be alternative procedures to investigate the nutritive value and ruminal degradability of feedstuffs, especially for those which are not suitable for *in situ* methods.

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